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Correlation between the experimental intestinal invasion and infection in poultry

Współzależność doświadczalnej inwazji i infekcji jelitowej u drobiu

The problem of the correlation between parasites and bacteria is hitherto not sufficiently solved. The relation which takes place between the parasitofauna and the bacterial flora was by various authors interpreted in various ways and was variously determined. Originally the opinion prevailed that parasites are the inevitable partners, as well as vectors of all infectious diseases. Significant is Blanchard's opinion expressed at the International Congress of Surgeons in Berne in 1904 in the words: „there are no infectious diseases of the alimentary tract without the presence of helminths, which open the way for bacteria”. Skrjabin in 1923 described the role of parasites in the following way: „invasion is a gate for the entrance of bacteria”.

The results of works of Weinberg (1907), Podjapolskaja and Dedova (1937) and next that of Gerbil'skij (1946) confirm the above theses only in appearance.

On the other side a number of authors deny the role of parasites as agents facilitating bacterial infection. As shown by their investigations, there can be observed rather a certain kind of a toleration of parasites towards the bacteria (Taylor et Purchase, 1931; Taylor, 1935; Kurzeja et Oleś, 1957; Stefański, 1958; Przyjałkowski, 1958).

It is very likely that in some cases we are dealing with an antagonistic action of substances excreted by worms, a fact to which attention is drawn by: Brumpt (1949), Jettmar (1952), Stefański (1955) and Emanuiloff (1958).

This does not refer to the possibility that bacteria may be transferred by larvae of parasitic worms actively penetrating the skin

(Smirnov et Kamalov, 1951; Stefański, 1958; Stefański, Majdan et Wertejuk, 1959).

In certain cases the worms may transfer, or even constitute a reservoir of viruses (Shope, 1943), in other, however, they play no serious role (Stefański et Żebrowski, 1958).

In some groups of diseases there can be observed a certain correlation between the parasitofauna and the bacterial flora present in the alimentary tract of hosts (Puchov et Milovzorov, 1934; Czapliński et Przyjałkowski, 1956); Iwańczuk, Macierewicz, Horbowska et Truchanowicz, 1958).

There are also known numerous clinical observations referring to the complications of various infectious diseases by helminthiasis. They are reported by: Bušuev (1892), Zilberberg (1913), Orlov (1930), Kosmačevskij (1938), Nemirovskij (1946), Murzin (1946) (cited after Podjapolskaja, 1954).

Studies conducted by: Ackert, Egerton et Hansen (1951), and next by Gerbilskij (1953) show that the parasitofauna exerts also an influence on the decrease of the resistance of the host to bacterial toxins.

According to observations made by Kotlan (1956) the invasion of larvae of parasitic worms may cause secondary infectious diseases by making active the facultative pathogenic bacteria present in the intestine of the host.

A complete view on the correlation taking part between the parasitic fauna and the bacterial flora is correctly presented by Stefański (1955) in the following thesis: "between the parasite and the bacterial flora coexisting in the proper host there develops in the way of evolution a certain relation, the outcome of which is a certain mutual tolerance, and sometimes even a mutual dependence".

The author's studies

It was aimed in this work to determine if there is a correlation between the course of ascaridiosis and typhus in poultry. For the experiments the mentioned pathological units were selected therefore, because on one side they are a very common feature in our domestic birds — particularly ascaridiosis, on the other side the infection with typhus takes place *per os*, therefore identically as the case is with ascaridiosis.

Material and methods

The experiments were run on 286 chickens (cocks) of the Sussex breed, at the age of 8 weeks.

By the determination of the age of chickens the following were taken into consideration: (1) greater susceptibility to the invasion of parasites in young birds at the age up to 3 months, and (2) greater susceptibility of older poultry to the typhus bacteria than in chickens.

The chickens used in the experiments were obtained from the State Zootechnical Institute in Czechnica and before the infection they were examined parasitologically and bacteriologically.

The flotation method of Fülleborn was employed for the parasitological examinations. In view of the stickiness and compactness of the faeces they were kept before centrifugation for 24 hours in the saturated solution of common salt. The results were always negative.

For bacteriological examinations the blood was collected from the wing vein and transferred onto bouillon with bile. On the second, third and eight day of cultivation the material was transplanted on solid selective media. No growth of *Salmonella gallinarum* was found. At the same time there were examined the faeces collected directly from the cloaca, whereby for the reproduction of bacteria the medium SF (acid sodium selenite) was used. Following 18 hours the material was transferred onto solid selective media. There was no growth of the bacteria of the *Salmonella* group. For control also 10 diagnostic dissections were made.

For the cultivation of the invasive larvae of *Ascaridia galli* the method of Cvetaeva (1954) was used. The eggs collected from the terminal sections of uterus of adult females were placed in physiological solution of saline in Petri dishes and transferred to a thermostat at the temperature 26—28°C. The physiological solution of saline was changed every day and its level in the dishes was no higher than 1 cm. The development of larvae was not uniform in view of the various degree of development of the eggs collected from the uterus. The first larvae appeared in the culture after 8—9 days. This is in accordance with Feoktistov's (1950) data, who reports that the development of larvae depending on the temperature lasts: at 17°C—25 days; at 20°C—17—18 days; at 25°C—9 days; at 30°C—7 days; at 35°—39°C—5 days.

For invasion (after Feoktistov) eggs of the age 22—25 days were used. According to the mentioned author the larvae, which develop longer, produce better effect following invasion. Every time before an invasion was intended to perform the larvae were examined for vitality. The eggs were placed on a slide and after heating the movements of the larvae were observed under the microscope.

The strain *Salmonella gallinarum* was obtained from the State Veterinary Institute in Puławy. It was store on the Dorset's egg medium.

The biochemical properties of the original strain were tested on media containing: lactose, sacharose, maltose, glucose, mannit, arabinose, inosite, xylose, dulcitate, ramnose, H_2S and on milk with lacmus. Preliminary agglutination test was performed with sera HM and OD.

For infection there was used a bacterial suspension in physiological saline of a 24 hours culture on agar with an addition of lactose and bromthymol blue. The number of bacteria in the suspension was determined according to Mc Farland's standard.

The chickens were invaded with worms by direct administration of a culture of invasive larvae, 0.5 ml in amount (that is about 400 eggs per head), using for this purpose a tube introduced into the crop. If the chickens received a different number of eggs, the number of eggs was counted by the use of a magnifying glass.

To infect the chickens with typhus they were given directly to the crop, partly filled up with food, 0.5 ml (about 300 millions) of the culture of typhus bacteria per head.

As regards the experimental infection of chickens with typhus bacteria there is in the literature no agreement of opinions. According to one group of authors the infection per os not always gives positive results (Nikiforova, 1951; Bushnell, 1949), however Tartakovskij (cited after Nikofova, 1951) was able in his experiments produce always infection of hens by feeding them with infected material.

The dose of the typhus bacteria which causes the disease was determined experimentally on 20 chickens.

In the course of the experiments there were conducted clinical observations and bacteriological examinations. Following the death of the chickens there were performed parasitological, bacteriological and anatomo-pathological examinations.

Bacteriological examinations of the faeces on the presence of

the typhus bacteria were conducted on the 15th and 25th day following the infection. After their death the carcasses were dissected and attention was turned to anatomo-pathological lesions, presence of worms and bacteriological examinations were conducted. Samples of the material from bile, liver and spleen were transferred onto the fluid media SF, and after 18 hours they were transplanted on agar media with an addition of lactose and bromthymol blue. The antigenic properties of the colonies were examined by the use of the agglutination test employing the diagnostic sera HM and OD. Strains which showed the characteristics of *S. gallinarum* were transferred on Dorset's egg media. After the termination of a series of examinations 5 strains of every experimental group were liberally selected from the isolated cultures and their biochemical and serological properties were studied. The results were compared with the results of the strain used for the infection.

The studies were conducted in 5 series and included:

- I — studies on the correlation between ascariidiosis and typhus;
- II — studies on the influence of invasion of helminths on the moment of infection with bacteria of typhus;
- III — studies on the number and size of migrating larvae in chickens only with ascariidiosis and in chickens suffering from ascariidiosis and typhus;
- IV — histopathological studies of the small intestine;
- V — studies on the influence of the extract of whole helminths and of the gonads of females on the growth of the typhus bacteria on liquid media.

Results of experiments

Series I

In the first series the following five groups were discerned:

group 1 — using a tube simultaneously were introduced into the crop a culture of *S. gallinarum* and eggs of *A. galli*;

group 2 — first a culture of typhus bacteria was administered and after 7 days eggs of *A. galli*;

group 3 — first the birds were invaded with helminths and after 10 day they were infected with typhus bacteria;

group 4 — first the birds were invaded with helminths and after 18 days with typhus bacteria;

group 5—controls: (a) infected with typhus bacteria, (b) invaded with helminths, (c) normal.

In the experimental groups 1, 2, 3 and 4 the number of chickens were 25 in each group, the control group (5) numbered 50 chickens.

Group 1

In this group the chickens received simultaneously helminths (about 400 eggs) and typhus bacteria (about 300 millions of bacteria).

Out of the total number of 25 infected chickens, 6 died and the remaining 19 were killed; 13 of them were killed on the 30th day, that is at the time when all the helminths are in the lumen of the intestine of the host; 5 of them were killed not before the 50th day to take dimensions of adult helminths.

The first clinical symptoms were observed on the 6th — 7th day following infection. They were manifested by loss of appetite, drowsiness and diarrhea. In the course of the progress of the disease there appeared paleness and dryness of the comb and wattles and swellings of the joints of the limbs. One week after the infection the temperature increased on an average by 1 — 2°C.

In the first four chickens, which died between the 8th — 11th day following the infection (No. 1, 11, 12 and 14), no helminths were found in the intestinal lumen, because the time for their development was too short. According to Cvet a e v a (1954) the larvae of the helminths remain in the mucous membrane from 17 — 18 days.

The following morbid lesions were found: widening and thickening of the duodenum, congestion of the initial sections of the jejunum, enlargement of the liver and spleen and considerable amount of dense bile in the gallbladder.

In the liver of the bird No. 14 there were found numerous, grey subcapsular necrotic foci. The post-mortem findings in the two remaining dead chickens were similar to those diagnosed in birds which were killed.

In the killed chickens helminths were found in numbers ranging from 68 to 193 (average about 170 per head). Post-mortem findings in this group of chickens were as follows: catarrh of the small intestine, enlargement of the liver and spleen, which were of dark-brown or brown-green colour, and enlargement of the gallbladder. The gallbladder filled up with dense bile. In some birds there were found necrotic foci under the capsule of the liver and under the pericardium. The heart muscle was pale and dull.

Bacteriological cultures, with the exception of the chicken No. 20, were positive.

In the chicken No. 20 post-mortem examination revealed the presence of helminths and slight hyperaemia of the small intestine. No other lesions were found.

Group 2

In this group the chickens were first infected with typhus bacteria and after 7 days with eggs of helminths.

Out of the total number of 25 chickens, used in the experiment, 7 died, 3 of them (No. 3, 9 and 22) before the invasion with helminths, 1 of them (No. 10) on the day of invasion, and 3 of them (No. 6, 12 and 14) over 10 day following invasion.

No clinical symptoms were observed in the bird which died on the 4th day following infection with typhus. Post-mortem findings: slight enlargement of the spleen and liver. Dark cherrecoloured comb. Culture positive. In chickens No. 3, 9 and 10 the post-mortem findings were: slight hyperaemia of the mucous membrane of the small intestine, enlargement of the spleen, liver and gallbladder. In the remaining birds submitted to slaughter the clinical symptoms and post-mortem findings were similar to symptoms and lesions observed in the first group. The number of helminths fluctuated from 132 to 210 (average 184 per head).

With the exception of the chickens No. 8 and 20 the cultures from all the remaining birds were positive. In birds, from which the cultures gave negative results on post-mortem examinations, no lesions typical to typhus were found.

Group 3

The chickens were first invaded with eggs of helminths and after 10 days they were infected with a culture of typhus bacteria.

In this group out of the total number of 25 chickens 7 died, 6 of them following infection with typhus and one on the day of infection. In the bird which died first (No. 22) post-mortem examination revealed only strong hyperaemia of the mucous membrane of the duodenum and the initial section of the jejunum (the mucous membrane looked as sprinkled with blood).

In birds which died later (No. 1, 3, 13, 16, 18 and 24) the exitus was preceded by a decrease of appetite and drowsiness. The post-mortem findings corresponded to the acute form of typhus. Positive bacteriological cultures were obtained.

In the remaining chickens, which were killed, the clinical symptoms and the post-mortem findings corresponded to the chronic form of typhus. All bacteriological cultures were positive. The number of helminths in the separate chickens (submitted to slaughter) fluctuated from 125 to 189, average 174 helminths per head.

Group 4

The chickens of this group were initially invaded with helminths and after 18 days infected with typhus bacteria.

In this group out of the total number of 25 chickens one died following the invasion with helminths and the ten remaining chickens following secondary infection with typhus. The number of helminths in the intestinal lumen of the 10 dead chickens fluctuated from 59 to 119. The clinical symptoms and the post-mortem findings corresponded to changes found in the course of typhus. Cultures — positive.

In the remaining 14 birds the number of helminths fluctuated from 141 to 191, an average of 172 helminths per a chicken. The post-mortem findings similar to the picture of chickens submitted to slaughter in the previous groups. Cultures from all chickens were positive.

Group 5

This group included control chickens: (a) infected with typhus bacteria; (b) invaded with helminths; (c) normal chickens.

Ad (a). Out of the total number of 20 chickens infected with typhus bacteria 4 died, the remaining chickens were killed after 30 days. In the fallen birds the following post-mortem lesions were found: hyperaemia of the mucous membrane of the duodenum, enlargement of the liver, spleen and gallbladder filled up with thick bile. In the remaining chickens the clinical symptoms and post-mortem findings corresponded to the chronic form of typhus. Bacteriological cultures, both from fallen birds and from chickens saughtered, were positive.

Ad (b). Out of 20 chickens invaded with eggs of helminths, one chicken died on the 12 day following invasion (strong hyperaemia of the duodenum and partly also of the jejunum); the remaining birds were directed to slaughter — 14 were slaughtered on the 30th day, the remaining 5 on the 50th day. The number of helminths fluctuated between 82 and 179, an average of 165 helminths per a chicken. In these chickens a slight hyperaemia of the small intestine was observed. Control bacteriological cultures from the liver, spleen and bile gave negative results.

Ad (c). Out of 10 normal chickens no one died during the observation period. Post-mortem findings and bacteriological examinations gave negative results.

Description of results of experiments of the I series

Numerical results of the experiments of the I series are collected and shown in the Table I.

During the invasion of the chickens in the separate groups into consideration was taken the life cycle of the parasite and first of all the moments of the penetration of larvae into the mucous membrane of the small intestine.

Basing on the works of Tugwell et Ackert (1952), the chickens of the first group were infected simultaneously with the helminths and typhus, because according to the authors' opinion the penetration of larvae starts already on the first day following invasion. In the second group, however, in order to observe the helminths in the course of the typhus, the birds were first infected with typhus, and secondarily after 7 days they received the helminths. In the third group the chickens were first invaded with eggs of the helminths and after 10 days they were infected with typhus bacteria, taking into mind that the penetration of the larvae into the interior of the mucous membrane reaches peak phase between the 10th and the 17th day after invasion (Tugwell et Ackert, 1952). Sadun (1949), too, is of the opinion that the maximal penetration of larvae takes place on the 11th day following the invasion and is associated with the highest mortality rate. Finally, in order to make observations on the course of typhus in chickens at the time when the majority of larvae penetrated into the mucous membrane, the birds of the 4th group were first invaded with eggs of helminths and secondarily after 18 days infected with typhus bacteria.

Analysing the results of studies in the I series, shown in the Table I, it can be observed that a larger percentage of mortal cases (40 chickens) is recorded after the primary invasion with the helminths and secondary (after 18 days) infection with the typhus bacteria (group 4). No essential differences were observed in the course of the disease between the groups 1, 2, 3 and 5a.

These results seem to indicate that the secondary infection with the typhus bacteria causes not sooner than after over ten days following invasion with helminths an exacerbation of the course of the disease (higher mortality). Simultaneous infection, or first an invasion with helminths followed after several days by an infection with typhus, or first an infection with typhus followed after

a week by an invasion with helminths; most likely does not increase the mortality rate in chickens and the disease runs a course similar to that observed in chickens suffering from typhus alone. This can be explained by the fact that mortal cases in chickens were caused to a major degree by the infection with typhus and when the infection coincided with a longer lasting process of ascaridiosis, and by the same coincided with lowered resistance of the chicken, the disease run in its first phase a more acute course — causing higher mortality.

Comparing the course of the typhus produced by artificial infection with the course of typhus in natural infections (compared with the literature) it should be pointed that artificial infection runs a milder course, what is evidenced by lower mortality rate.

Stryszak (1947) reports that during the enzootic typhus observed in chickens in 1942 — 1944 in the Warsaw Palatinate the mortality rate reached up to 80% and the duration of the disease was on the average 2 — 5 days. The chronic form occurred not frequently and extended over several months.

In my experiments I observed, however, after artificial infection with typhus more commonly the chronic form.

Comparing the intensity of invasion in chickens after mixed infection with the intensity of occurrence of parasites in birds invaded with worms only, it should be stressed, that the difference is minimal. And so: in the groups 1, 2 and 4 a somewhat larger number of helminths was found than in the control birds, then, however, in chickens of the 3 group there are no differences whatsoever.

There can be observed, however, a low percentage of detected helminths (68 — 210) in relation to the number of eggs used for the invasion ($\pm$ 400). This phenomenon is the result of the increasing of the resistance with the coming age, a fact stressed also by other authors.

Measurements were also taken from adult helminths (males and females) and no differences were detected in size between the 1st, 2nd, 3rd, 4th and 5th b. groups. The dimensions obtained correspond to the dimensions of the helminths given by Klimeš et Švec (1955) (cited after Ackert, 1931).

The strains of *S. gallinarum* cultivated from the invaded chickens were, after the termination of the series of investigations,

examined biochemically and serologically. The results obtained in comparison with the corresponding results of the original strain proved agreement of biochemical and serological properties.

Series II

In this series studies were conducted to find out whether the invasion with helminths accelerates the infection with the typhus bacteria in small doses. The chickens were simultaneously infected with subinfectious doses of the typhus bacteria (0.1 — 0.5 ml) and with eggs of helminths, increasing the dose of the number of eggs from 100 to 500 per head in the separate groups, whereby the dose of the typhus bacteria remained the same.

The experiment was run on 5 groups, infecting in every group 8 chickens, 5 of which received simultaneously a culture of the typhus bacteria and eggs of helminths; the 3 control chickens received the typhus bacteria only.

The course of the experiments in the separate groups was as follows:

group 1 — the chickens received simultaneously 0.1 ml of typhus bacteria culture and eggs of helminths, increasing their number from 100 — 500 per head. The 3 control birds received only 0.1 ml of the suspension of typhus bacteria. Because after 10 days following the invasion (at the time when the largest number of the larvae penetrates into the mucous membrane) no pathological symptoms were observed and the previously conducted control bacteriological examination of the faeces gave negative results, the chickens were again infected with the same dose of typhus. The chickens were directed to slaughter on the 5th day following the repeated infection. Postmortem examination revealed no lesions typical to typhus. In the control chickens the post-mortem findings were negative. All bacteriological cultures were negative.

group 2 — the chickens received simultaneously 0.2 ml of typhus bacteria culture and eggs of helminths (from 100 to 500). The control chickens received only the typhus bacteria. After 10 days the infection with typhus was repeated. The results were similar to those obtained in group 1.

group 3 — the chickens received 0.3 ml of the suspension of the typhus bacteria per head, further procedure as in the groups 1 and 2. Results of infection negative.

group 4 — the dose of the typhus bacteria was increased to 0.4 ml per head and the chickens received simultaneously eggs of helminths (from 100 to 500). After the repeated infection after 10 days with the same dose of the typhus bacteria (0.4 ml), in one chicken directed to slaughter after 15 days there was observed on post-mortem examination slight enlargement of the spleen, but the bacteriological cultures from this bird and the remaining ones were negative.

group 5 — this group of chickens was infected with a dose of 0.5 ml of the culture of typhus bacteria that means with a dose used in the experiments of the I series to produce this disease. The number of administered eggs was as previously, increasing to the separate bird the number from 100 to 500. On the 6th day following infection the chicken which received 0.5 ml of the culture of the typhus bacteria and 300 eggs of helminths died. The post-mortem findings were typical to typhus. Bacteriological examinations were positive. In the remaining birds there were observed on the 6th and 7th day decrease of appetite and drowsiness. The temperature of the body was higher. On the 10th day the chickens were directed to slaughter. Anatomopathological lesions and bacteriological examinations confirmed infection with typhus.

Description of results of experiments of the II series

As shown by the experiments conducted in the II series neither the invasion of helminths nor the intensity of invasion exert any influence on the moment of infection with typhus when using the subinfectious dose of *S. gallinarum*.

It was possible to produce the disease not till in the 5th group of experiments, where the dose used for infection was 0.5 ml, that is such a dose, as was used when the birds were infected regardless of the presence of helminths. In this group typhus was found both in the experimental and in the control chickens.

The results of this series of studies corroborate the results of a number of authors (Taylor et Purchase, 1931; Taylor, 1935; Stefański, 1956; Przyjałkowski, 1958, and others), who also failed to succeed to increase the susceptibility of experimental animals to bacterial infection by the „assistance” of parasitic invasion.

Series III

It was aimed in the studies conducted in this series to find out the differences in the number and size of the migrating larvae of *Ascaridia galli* in chickens invaded only with helminths and in chickens invaded with helminths and infected with typhus.

In this experiment two groups of chickens were used. One of them (21 chickens) received only eggs of helminths, the second received simultaneously the eggs of helminths and the culture of the typhus bacteria. The number of chickens in the second group was 25. Bearing in mind that the greatest number of helminths larvae start migrating from the 11th up to the 17th day after invasion, the birds were at that time directed to slaughter, of every group 3 chickens daily. The larvae were collected from the lumen of the intestine by the hydraulic method; the larvae inserted in the mucous membrane were collected by the method of digestion (both methods according to Tugwell et Actert, 1952).

1. The hydraulic method: the intestine was separated from the stomach and mesentery, divided into 30 cm sections and washed under pressure with warm water. The material obtained in this way was preserved under heat using 75° alcohol with an addition of 5% of glycerol.

2. The method of digestion: digestion was produced in an apparatus specially adapted to this purpose. Two containers, 1 litre volume each, were placed in a water bath of a constant temperature 38° (thermoregulation). In the containers filled with aqueous solution of 0.5% hydrochloric acid and 1% pepsin the previously washed intestine was placed for a period of 3 — 4 hours (depending on the volume). During the process of digestion the contents in the containers were continually mixed by the use of glass rods electrically set in motion. At the end of digestion the intestine was removed and the liquid left till the sedimentation of the larvae on the bottom of the container. Subsequently using a glass tube of the shape of the letter J and a sucking pump the liquid was withdrawn from above the sediment till a level of 3 cm from the bottom was left. The sediment was several times made to settle and it was examined by using a binocular magnifying glass. The larvae collected in this way were preserved under heat using 75° alcohol with the addition of 5% of glycerol. Measurements of larvae were taken under the microscope. Results of the experiments are shown in the Table II.

Table II
Results of the III series

Group	No. of chickens	Manner of invasion	Day of killing	Larvae in lumen of intestine		Larvae in mucous membrane		Aver. no. of all larvae
				aver. no.	aver. length in mm	aver. no.	aver. length in mm	
1	3	typhus & nematodes nematodes only	11	33	3.3	86	2.2	119
2	3		11	28	3.5	79	2.1	107
1	3	typhus & nematodes nematodes only	12	19	4.0	119	3.0	141
2	3		12	19	4.7	138	3.4	157
1	3	typhus & nematodes nematodes only	13	34	4.1	116	3.5	146
2	3		13	17	4.3	132	3.6	150
1	3	typhus & nematodes nematodes only	14	23	5.6	152	3.8	175
2	3		14	19	5.2	152	3.7	171
1	3	typhus & nematodes nematodes only	15	12	5.3	117	3.8	163
2	3		15	28	5.2	102	4.0	130
1	3	typhus & nematodes nematodes only	16	17	6.1	143	4.1	160
2	3		16	32	6.7	105	4.5	137
1	3	typhus & nematodes nematodes only	17	69	6.8	72	4.2	142
2	3		17	49	7.1	55	5.4	104

Description of results of experiments of the III series

Tabular arrangement of the results is represented in Table II.

As evident in the Table II on the average in the chickens of the first group somewhat more larvae were found than in the second group. The slight differences in the length of larvae in the separate groups are not essential and remain within the limits of normally occurring fluctuations in the development of the larvae. For this reason they cannot be taken into consideration.

However, certain differences found between the size of the larvae from the intestinal lumen and the larvae from the mucous membrane are, according to Tugwell et Ackert (1952), caused by fact that the larvae present in the mucous membrane find more unfavourable conditions for their development. The authors stress also that the moulting of the larvae in the mucous membrane are delayed too. The second moulting, which normally takes place between the 6th — 8th day in the intestinal lumen, may be delayed in the mucous membrane and protracted from 8 — 17 days.

Series IV

The studies in this series of experiments aimed to compare the lesions in the mucous membrane of the small intestine in chickens with ascariidiosis with those found in chickens affected with ascariidiosis and typhus taking as a basis of these studies the histopathological picture.

In this experiment 20 chickens arranged in two groups were used:

group 1—14 chickens received about 1000 eggs of helminths per head and after 8 days they were infected with a suspension of typhus bacteria 0.7 ml per chicken. Both the number of eggs and bacteria were augmented to obtain satisfactory results. One half of the birds were directed to slaughter on the 11th day following the invasion with helminths, the remaining — on the following day.

group 2—6 chickens were invaded with eggs of helminths (about 1000 per head) and after 11 days they were directed to slaughter.

The carcasses of chickens of the two groups were submitted to post-mortem examination. From the sections of the small intestine affected by lesions (hyperaemic parts of the duodenum and jejunum), fixed in 10% formol or Bouin's fluid, serial histopathological paraffin sections were made.

The sections were stained by using the following methods: (a) Löffler's methylene blue, (b) Ehrlich's hematoxylin with eosine, and (c) azan in Heidenhein's modification*.

Description of results of experiments of the IV series

The histopathological picture of preparations made from the intestines of chickens of the 1st group (chickens invaded with helminths and infected with typhus) was similar to the picture of intestines of chickens invaded with helminths only (chickens belonging to the 2nd group) (Phot. 1 — 4).

It was frequently observed in the microscopic picture that in places of the invasion of the larvae of parasites the superficial epithelium of villi and of glandular ducts was injured. The goblet cells of the epithelium in the neighbouring parts were increased in number and secreted a larger content of mucin. The nuclei of the superficial epithelium showed in numerous places signs of hyperplasia. In the reticular structure of the tissue of the villi, particularly close to the parasites, the number of leucocytes was increased. Sometimes there were observed in the glandular layer dilated ducts filled up with coagulated proteins and desquamated cells of the epithelium. There was also lymphocytary hyperplasia in the lymph nodes. In some places the multiplied cells of the lymph nodes formed clear pegs which seem to grow into the villi. The observed apparent thickening of the villi was undoubtedly caused by the contraction of the smooth muscles effected by the fixative agents.

In the microscopic picture of the histopathological preparations of the intestines of chickens invaded with helminths and infected with typhus the presence of the typhus bacteria around the larvae situated in the mucous membrane could not be proved. This certainly does not exclude the possibility that the bacteria could be drawn by the migrating larvae into the interior of the mucous membrane. However, it appears to be too difficult to prove fact in an experiment.

It should be stressed that the anatomopathological picture of the mucous membrane of the intestines of the two groups showed some differences. In birds of the first group the hyperaemia of the mucous membrane was more strongly marked and extended over a greater part of the intestines (duodenum, jejunum and even the

* Histopathological preparations were made by Master Zofia Mróz.

initial sections of the ileum). In the second group, however, the hyperaemia of the mucous membrane was limited to the duodenum and the initial sections of the jejunum; the character of the hyperaemia was in this case rather that of punctate haemorrhages. In the two of dissected birds an increased secretion of mucin was found.

The anatomopathological picture of the second group was similar to the picture presented by Kądziołka (1956).

Series V

In this series studies were conducted to find out the influence in vitro of the extract from the helminths in toto and of the extract of the gonads of the females (from eggs) on the growth of *S. gallinarum* on liquid media. The helminths after they were washed in distilled water were dried in a thermostate at the temperature 37°C. After thorough crushing, preceded by accurate drying, a powdered substance was received. This was extracted in physiological solution (in proportion 1 g of the substance to 100 ml of the solution), by leaving the suspension in a refrigerator at the temperature plus 4°C for 8 days and shaking the suspension several times daily. After passing the suspension through a filter paper the filtrate was sterilized in a water bath at the temperature 55°C for 45 minutes. The sterility of the extract was testified bacteriologically. The extract from the gonads collected from mature females was prepared in the identical way.

The studies were conducted in three groups:

group 1 — to the test tubes containing each 10 ml of liquid medium (meat bouillon) a solution of the extract of whole helminths was added in the following amounts: to the first — 3 ml, to the second — 2 ml, to the third — 1 ml, to the fourth — 0.5 ml. Next to every test tube was given 1 drop of the 24 hours bouillon culture of the bacteria of typhus.

group 2 — procedure identical as in the 1st group only the extract of the whole helminths was substituted by the extract of the gonads from females.

group 3 — controls: bouillons with bacteria of typhus without the extracts.

The test tubes were subsequently placed in a thermostat at the temperature 37°C, for 24 and 48 hours.

Description of results of experiments of the V series

After 24 and 48 hours no differences were found between the groups, 1st, 2nd and 3rd. The growth was in all of them intensive.

The above experiments indicate that neither the extracts of the whole helminths nor the extracts of the gonad of females exert any visible influence on the growth of the strain of *S. gallinarum* on liquid media during the period of 24 to 48 hours of cultivation.

Similar tests conducted in vitro by Jettmar (1952) with the fluid from the body cavity of *Parascaris equorum* showed also the lack of any influence of this fluid on the gram-negative bacteria.

Discussion and conclusions

From the experiments conducted in 5 series it can be concluded that between ascaridiosis and typhus in chickens there is no direct correlation. This is supported by the fact that the invasion plays no role on the infection of chickens with a subinfectious dose of a culture of the typhus bacteria, by the lack of any differences during the time of the development of helminths and by the lack of any influence in vitro of the extract of helminths on the culture of the strain of *Salmonella gallinarum*.

Summing up the above results it can be stated that a certain correlation that seems to be between the course of typhus and the degree of ascaridiosis in chickens with mixed infection is most likely caused by the lowered resistance of the host.

From the above experiments the following conclusions are drawn:

(1) In chickens invaded first with helminths and after over ten days with typhus the course of the disease runs a more acute form than in chickens infected with typhus only.

(2) In chickens simultaneously invaded with helminths and infected with typhus, or first with helminths and after several days with typhus, or first with typhus and after a week with helminths, the disease runs similarly as in chickens infected exclusively with typhus.

(3) The invasion with helminths exerts no influence on the infection of chickens with a subinfectious dose of the culture of the typhus bacteria.

(4) The number of larvae, as well as the number of mature helminths are somewhat greater in chickens infected with typhus than in chickens free from typhus, invaded by the same number of eggs of helminths.

(5) No differences are found in the size of larvae of helminths present in the lumen, as well as in the mucous membrane of the small intestine of the chickens with typhus and those with ascari-diosis alone.

(6) No differences were found in the size of mature helminths of chickens with typhus and those with ascari-diosis alone.

(7) The microscopic picture of the histopathological preparations of the intestines of chickens invaded with helminths and infected with typhus is similar to the microscopic picture of the intestines of chickens invaded with helminths alone.

(8) *Extracts of whole helminths and extracts of gonads of females* exert in vitro no influence on the growth of the strain of *Salmonella gallinarum* on liquid media.

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STRESZCZENIE

Celem niniejszej pracy było ustalenie współzależności między przebiegiem glistnicy (*Ascaridia galli*) i tyfusu (*Salmonella gallinarum*) u drobiu. Do doświadczeń wybrano właśnie te jednostki chorobowe, gdyż są one z jednej strony dość pospolitym zjawiskiem u naszego ptactwa domowego — zwłaszcza glistnica, z drugiej zaś strony zakażenie tyfusem odbywa się per os, a więc identycznie jak przy glistnicy.

Doświadczenie przeprowadzono na 286 kurczętach (kogutkach) rasy Sussex w wieku 8 tygodni.

W badaniach uwzględniono 5 serii:

- I — badania nad współzależnością przebiegu glistnicy i tyfusu;
- II — badania nad wpływem inwazji na moment zakażenia pałeczkami tyfusu;
- III — badania nad określeniem ilości i wielkości migrujących larw u kurcząt tylko z glistnicą oraz u kurcząt z glistnicą i tyfusem;
- IV — badania histo-patologiczne jelita cienkiego;
- V — badania wpływu wyciągu z całych glist i z gonad samic na wzrost pałeczek tyfusu na pożywkach płynnych.

Z przeprowadzonych badań wynika, że między glistnicą a tyfusem u kurcząt nie występuje bezpośrednio współzależność. Dowodem tego jest brak wpływu inwazji na zakażenie kurcząt subinfekcyjną dawką hodowli pałeczek tyfusu, brak jakichkolwiek różnic w czasie rozwoju glist, jak również brak wpływu in vitro wyciągu z glist na hodowlę szczepu *Salmonella gallinarum*.

Resumując powyższe wyniki należy stwierdzić, że pewna zależność, jakiej można by się dopatrywać między przebiegiem tyfusu a stopniem glistnicy u kurcząt z zarażeniem mieszanym, jest prawdopodobnie spowodowana obniżoną odpornością żywiciela.

Z przeprowadzonych badań można wyciągnąć następujące wnioski:

1. U kurcząt zarażonych najpierw glistami a po kilkunastu dniach tyfusem, przebieg schorzenia jest ostrzejszy niż u kurcząt zakażonych samym tyfusem.

2. U kurcząt zarażonych równocześnie glistami i pałeczkami tyfusu, lub najpierw glistami a w kilka dni potem tyfusem, czy też najpierw tyfusem a po tygodniu glistami, schorzenie przebiega podobnie jak u kurcząt zakażonych wyłącznie tyfusem.

3. Inwazja glist nie wpływa na wywołanie zakażenia u drobiu subinfekcyjną dawką hodowli pałeczek tyfusu.

4. Liczba larw oraz liczba dojrzałych glist jest nieco większa u kurcząt zakażonych tyfusem niż u kurcząt bez tyfusu, zarażonych taką samą ilością jaj glist.

5. Nie stwierdzono różnic w wielkości larw glist znajdujących w świetle i błonie śluzowej jelita cienkiego u kurcząt z tyfusem i u kurcząt z samą glistnicą.

6. Nie stwierdzono różnic w wielkości dojrzałych glist u kurcząt z tyfusem i u kurcząt z samą glistnicą.

7. Obraz mikroskopowy preparatów histo-patologicznych jelit kurcząt zarażonych glistami i tyfusem jest podobny do obrazu mikroskopowego jelit kurcząt zarażonych wyłącznie glistami.

8. Wyciągi z całych glist oraz z gonad samic nie wpływają in vitro na wzrost szczepu *Salmonella gallinarum* na pożywkach płynnych.

EXPLANATION OF PLATES

PLATE I

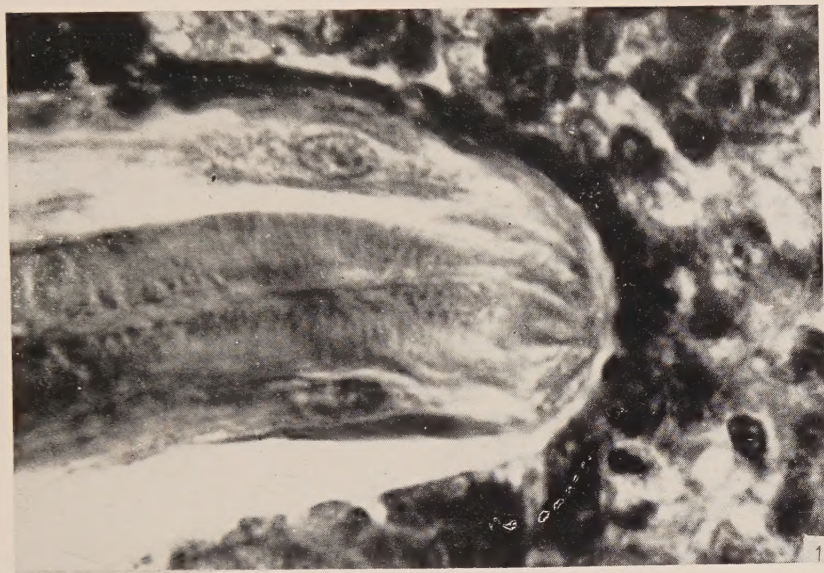
Phot. 1. Group 1 — Larva in mucous membrane of small intestine. Damage of the epithelium of gland duct and multiplication of leucocytic elements are seen. Obj. 100, ocul. 6.3 X.

Phot. 2. Group 1 — Larva in mucous membrane of small intestine. At the place of invasion of larva the wall of gland duct deprived of epithelium, beneath epithelium is present. Multiplication of leucocytic elements. Obj. 10, ocul. 6.3 X.

PLATE II

Phot. 3. Group 1 — Larva in the gland duct. Considerable damage of the epithelium of gland duct and excessive multiplication of leucocytic elements are seen. Obj. 10, ocul. 6.3 X.

Phot. 4. Group 2 — Larva in the gland duct. Damage of the epithelium of gland duct and multiplication of leucocytic elements are seen. Obj. 10, ocul. 6.3 X.



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auctor phot.

